BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

#34

PATENT

Attorney Docket No. 16243-1-5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Richard H. Tullis

Serial No.: 08/078,768

Filed: June 16, 1993

For: OLIGONUCLEOTIDE THERAPEUTIC AGENT AND METHODS OF MAKING SAME

Examiner: J. Martinell

DECLARATION OF JERRY L. RUTH

Box AF
Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Dr. Jerry L. Ruth, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true. The Exhibits attached hereto are numbered 1-9. They are each incorporated herein by reference.

2. I have previously submitted a declaration in support of this patent application. My scientific credentials are presented therein.

3. As I understand the Examiner's remaining rejection, he believes that the pending claims should be restricted to the phosphotriester-modified nucleic acids that are stated as a preferred embodiment representing a class of stabilized nucleic acids for *in vivo* applications. The Examiner reasons that the pending claims inclusive of *in vivo* uses are too broad. More specifically, the Examiner states that no other suitable nucleic acid analogs were available as of the filing date in October of 1981, that unmodified nucleic acids would not be adequately stable to have biological activity under *in vivo* conditions, and that the means for administering the antisense molecules would require undue experimentation.

The purpose of this declaration is to address each of the above issues. More specifically, I will explain: (1) that as of the priority filing date, those of skill were aware of other analogs of nucleic acid that were modified to enhance stability against nuclease activity and which were suitable for *in vivo* use and that those of skill would have recognized from the Applicant's disclosure that these known analogs of nucleic acids were useful in the invention; (2) that the stability of natural (unmodified) nucleic acid under *in vivo* conditions is sufficient to permit the observation of its biological activity, *i.e.*, to inhibit expression of specific protein; and, (3) given the level of skill in the art, there is nothing but routine experimentation involved in the *in vivo* use of the claimed method.

A. PRIOR TO THE FILING DATE OF THE PARENT APPLICATION ON OCTOBER 23, 1981, A NUMBER OF STABILIZED NUCLEIC ACID ANALOGUES WERE AVAILABLE FOR USE IN THIS INVENTION.

1. The alkylphosphotriester DNA analogs described in the application as an example of a stabilized oligonucleotide were described in the literature in 1974 by Miller *et al.* (A1). These analogs have a phosphate bearing four oxygens, three of which are substituted with carbon-based substituents. The following discussion presents other references that describe chemically modified nucleic acids that were available prior to October of 1981 and were used intracellularly. Collectively, they present uncontestable evidence that a variety of stabilized nucleic acids were

known and available for use in the claimed invention as of the original filing date.

A second chemically modified nucleic acid was the methylated ribonucleic acids described by Befort *et al.*(1974). Befort is already of record as reference A27. In Befort, the authors reported uptake of their stabilized RNA into fibroblasts and the subsequent inhibition of viral multiplication. The stabilized nucleic acid was a methylated RNA that complemented a portion of the viral genome.

In Tennant *et al.* (1974), the authors describe the *in vivo* effects of an alkylated homopolymer of ribonucleic acid on virally induced oncogenesis. Tennant is already of record as reference A47.

In Kunkel *et al.* (Exhibit 1), *P.N.A.S. USA* 78(11):6734 (1981). The authors describe work conducted and published before 1981 using thio-substituted deoxynucleosides. On column 2 of page 6734, the authors describe that their analogs were previously reported as incorporated into oligonucleotides using DNA polymerase and nuclease resistant.

Finally, Miller *et al.* reported on the *in vivo* effects of a DNA analog in March 1981. This reference is already of record as A2. Attached to this declaration as Exhibit 2 is a true copy of the Medline abstract entry for this reference. The entry clearly identifies its publication date as March 1981, seven months before applicant's filing date. The analog described by Miller in 1981 was an alkyl phosphonate which differs from the phosphotriester of their earlier work by the direct attachment of the alkyl substituent to the phosphate. The Examiner is asked to review page 1879, second column, where a discussion of the intracellular half-lives of the phosphonates and the triesters are compared.

Thus, it is clear that as of the priority filing date of the present application, those of skill would have understood the applicant's reference to stabilized nucleic acid to have included more than the phosphotriester compounds that were specifically identified. Moreover, and because the use of stabilized nucleic acids was a mere example in a universe where both stabilized and natural oligonucleotides would function to downregulate expression of protein under *in vivo* conditions, it was, in my opinion, unnecessary to identify for those of skill all the stabilized nucleic acids that were available for use in the invention as of

October of 1981.

2. It is my understanding that the Examiner was concerned that the specification as filed would not have suggested that the nucleotide analogs described in the above references were useful in the invention. There are several objective reasons, why those of skill in 1981 would have understood that the text of the specification, i.e., page 4, lines 8-13 and claims 29-33 of the original specification was referring to the above identified body of knowledge.

The above referenced text of the applicant's disclosure states:

> The preferred oligonucleotide ... , for increased stability, may be transformed into a more stable form, such as a phosphotriester form, to inhibit degradation during use.

Original claims 29 and 32 recite stable forms of olignucleotides that inhibit degradation by organisms and claims 30 and 33 recite phosphotriester forms. The applicant clearly is teaching that stable, nuclease resistant forms of nucleic acid which can duplex to target nucleic acid are preferred forms of the oligonucleotides useful in the invention. Even presuming that those of skill were unaware of the above body of knowledge, to the best of my knowledge, there was no other body of knowledge to which the applicant could have been referring.

Having explained that there was no other body of knowledge that might have been confused with the above reference body of knowledge, it is simply a matter of establishing that one of skill would had the skill to locate the above references. The above references are representative of a significant body of work involving stabilized nucleic acids for understanding enzyme mechanics, transcription studies, for evaluting cellular uptake of nucleic acid and for medical uses. For the Examiner to maintain that those of skill would not have known of the above references or not have been able to find the above references is contrary to the way scientists work and contrary to my understanding of how the Patent Office establishes obviousness. The phosphotriester reference in the original application would have lead one of skill directly to Dr. Paul Miller's work and thus to other analogs. Dr. Miller's published work involved both phosphotriester and phosphonates analogs. Anyone familiar with Dr. Miller's work would have known

of analogous work by Dr. Fritz Eckstein using thio-substituted nucleic acid. Even undergraduates were being taught in 1981 that methylation was a key modification to nucleic acid for the purpose of increasing its half-life. In addition, the Examiner is asked to review the work of Dr. Summerton in 1978. This reference is already of record (A33). At page 89, Dr. Summerton summarized the art of modified nucleic acids for inhibiting viral replication and specifically addressed degradation problems. Among the modified nucleic acids taught by Summerton as useful as *in vivo* antiviral agents were the methylated oligonucleotides, thio-substituted nucleic acids as well as the modified olignucleotides of Miller.

Finally, as one of skill in 1981, I can state unequivocaly that I would have fully understood what nucleic acids analogs were being referred to by the applicant in his original application. For the above reasons, I am of the opinion that the Examiner's concern regarding whether one of skill would have understood the applicant's teaching to be inadequate to identify other modified nucleic acids is groundless.

B. UNMODIFIED RNA AND DNA HAVE A HALF-LIFE *IN VIVO* THAT IS SUFFICIENT TO PERMIT ITS UPTAKE INTO CELLS.

The Examiner questions whether unmodified nucleic acid will actually survive under *in vivo* conditions for a sufficient length of time to actually be taken up by the cells. There are numerous studies that should convince the Examiner that his concerns are without foundation. Unmodified nucleic acid does survive in the body for a fairly long period. While its susceptibility to degradation make more stable forms of nucleic acid a preferred embodiment, susceptibility to degradation does not render unmodified nucleic acid useless. It is merely a matter of dosage with unmodified nucleic acid requiring higher amounts and/or longer administration to see the equivalent effects of stabilized nucleic acid.

While most articles report on the use of DNA, unmodified RNA will also survive under *in vivo* conditions. In Michelson *et al.* (1985) "Poly(A)·Poly(U) as Adjuvant in Cancer Treatment Distribution and Pharmacokinetics in Rabbits

(42082)," *Proc. Soc. Exp. Biol. & Med.* 179:180-186 (Exhibit 3). In Michelson *et al.*, the authors describe the half-life of synthetic polyribonucleotides as measured in days. Its uptake into cells was also reported on page 184, 1st Col. A second report of long term survival of RNA appeared in Wolff *et al.* (1990) "Direct Gene Transfer into Mouse Muscle in Vivo," *Science*, 247:1465-1468 (Exhibit 4). In Michelson, purified RNA and DNA were simply injected into the muscle of mice and their respective gene products measured. The authors clearly state in their abstract that, "protein expression was detected in all cases and **no special delivery system was required....**"

Reports involving the use of purified DNA are more numerous than of RNA. Illustrative reports of DNA expression of plasmids directly injected into animals are provided in Lin *et al.* (1990) "Expression of Recombinant Genes in Myocardium In Vivo After Direct Injection of DNA," *Circulation*, 82:2217-2221 and Wolff *et al.* (1992) "Long-Term Persistence of Plasmid DNA and Foreign Gene Expression in Mouse Muscle," *Human Mol. Genet.* 1(6):363-369 which are Exhibits 5 and 6, respectively. The DNA may be linear or circular (see Exhibit 4 at page 368, 2nd Col.)

C. UNMODIFIED ANTISENSE OLIGONUCLEOTIDES HAVE BEEN DEMONSTRATED TO BE USEFUL IN A VARIETY OF DIFFERENT ORGANS.

Naked, natural phosphodiester, antisense oligodeoxynucleotides have been reported as sufficiently stable to downregulate gene expression when directly injected into an animal. For example, Phillip *et al.* (1994) "Antisense Inhibition of Hypertension: A new strategy for Renin-Angiotensin Candidate Genes," *Kidney Intern.*, 46:1554-1556 (Exhibit 7) reports on the direct injection of an antisense DNA (unmodified) for reducing hypertension in mice. The DNA was merely injected into the mouse carotid artery using a saline solution.

Others have reported that antisense DNA will work when directly injected into the brain. For example, in Akabayashi *et al.* (1994) *Mol. Brain Res. 21:55-61*, the authors dissolved the antisense DNA in saline and simply injected it into the

brain to inhibit production of a neuropeptide (Exhibit 8). At page 56, 1st Col., the authors state that theirs is the third such report.

As stated above, the use of stabilized DNA was merely a preferred embodiment. The use of unmodified DNA was less preferred, but similar results could be achieved by merely using more DNA or RNA to accommodate instability. Given the level of skill of those practicing molecular biology, this is an intuitively apparent solution to an obvious problem. The use of high levels of DNA is described in Exhibit 9, Hijya *et al.* (1994) *Proc. Natl. Acad. Sci., U.S.A.* 91:4499-4503. Hijya *et al.* report on the use of an unmodified phosphodiester oligonucleotide for controlling the expression of a gene which is involved in skin cancer. The authors applied the antisense oligonucleotide via a subcutaneous route and used constant-infusion pumps to ensure that the oligonucleotide was adequately administered.

D. THERE IS NO UNDUE EXPERIMENTATION INVOLVED IN THE ADMINISTRATION OF ANTISENSE OLIGONUCLEOTIDES.

The level of skill of those in the art of antisense technology is quite high. Most of the artisans are like myself and hold doctorates in a relevant biological science. To achieve a measurable downregulation of protein expression, one need only contact the target cells with an adequate amount of antisense oligonucleotides. The infusion techniques are conventional and were fully known in 1981. The technique is merely the injection of a saline solution containing the antisense oligonucleotides into the appropriate organ. There is simply no basis to conclude that such a experimental step was anything but routine and intuitively apparent to those of skill.

In summary, the relative stability of unmodified antisense oligonucleotides compared to stabilized oligonucleotides does not render the *in vivo* use of unmodified DNA or RNA without utility for the purpose of downregulating protein expression. The attached Exhibits 3-9 clearly document to one of skill that the claimed methods are operable under *in vivo* conditions. Furthermore, there is

nothing beyond routine experimentation required to administer the antisense oligonucleotides under *in vivo* conditions and detect a downregulation in the expression of a specific protein.

The declarant has nothing further to state.

Dated: 7 April 1995 By: Jerry L Ruth

Dr. Jerry L. Ruth

Attachments: Exhibits 1-9